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Sweet Sorghum as Feedstock for Ethanol Production: Enzymatic Hydrolysis of Steam-Pretreated Bagasse

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Abstract Sweet sorghum is an attractive feedstock for ethanol production. The juice extracted from the fresh stem is composed of sucrose, glucose, and fructose and can therefore be readily fermented to alcohol. The solid fraction left behind, the so-called bagasse, is a lignocellulosic residue which can also be processed to ethanol. The objective of our work was to test sweet sorghum, the whole crop, as a potential raw material of ethanol production, i.e., both the extracted sugar juice and the residual bagasse were tested. The juice was investigated at different harvesting dates for sugar content. Fermentability of juices extracted from the stem with and without leaves was compared. Sweet sorghum bagasse was steam-pretreated using various pretreatment conditions (temperatures and residence times). Efficiency of pretreatments was characterized by the degree of cellulose hydrolysis of the whole pretreated slurry and the separated fiber fraction. Two settings of the studied conditions (190 °C, 10 min and 200 °C, 5 min) were found to be efficient to reach conversion of 85–90%.

Keywords Sweet sorghum · Ethanol fermentation · Lignocellulose · Steam pretreatment · Enzymatic hydrolysis

Introduction

Sweet sorghum (*Sorghum bicolor* var. *saccharatum*) has a great potential as an energy crop [1]. It belongs to the C_4 family with a high photosynthetic activity and drought tolerance; therefore, it can be cultivated in almost all temperate and tropical climate areas [2]. This plant requires less fertilizer and water compared to other sugar crops. Sweet sorghum has

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been used for the production of edible syrup in North America for over 100 years [3]. A wide range of processes can apply this raw material as feedstock. In most cases, sweet sorghum juice is extracted from the fresh stem, then the liquid fraction and the remaining solid residue—bagasse—are processed separately.

There are several possibilities to convert sweet sorghum juice to a highly valuable product. The earliest use of this fraction was making white sugar from the liquid. This process results in a by-product, molasses, which is a viscous liquid containing high amount of sugars [4]. Nowadays, molasses is used as carbon source in the fermentation industry. Sweet sorghum juice itself can be used for ethanol production as well [5], and it was reported to be an ideal substrate for the production of gaseous biofuels such as hydrogen [6]. Sweet sorghum tends to deteriorate after harvest; thus, the main problem of juice processing is the microbial instability of the liquid. Several studies focused on increasing the storability of the extracted juice and the chopped stalk. The most suitable techniques were as follows: (1) evaporating the juice to a 60% syrup [3], (2) ensiling the chopped stalks in presence of 0.5% formic acid, and (3) enzyme-assisted ensiling of the stalk [7].

As the juice, sweet sorghum bagasse has also several ways of utilization. In contrast to sweet sorghum juice, which can be used in the food industry, the bagasse fraction has only non-food utilization alternatives. Earlier, it was used as animal feed or as soil fertilizer after composting with other wastes [1]. Nowadays, bagasse is mainly used for energy production by combustion [8]. The main problem with biomass combustion is the high ash content, which can cause slagging, corrosion, or fouling [9]. Antonopoulou et al. [6] used the remaining solids after the extraction process and evaluated the methane production. Furthermore, sweet sorghum bagasse was found to be a remarkable raw material for the paper industry, yielding high-quality pulp [10]. The most promising future utilization of bagasse is cellulose-based ethanol production, while the residual solids (mainly lignin) can be burned to provide heat and power. Hydrolysis of the cellulose and hemicellulose fractions can be catalyzed by acids or cellulolytic enzymes. Enzymatic process needs a pretreatment step to increase the susceptibility of the cellulose, which can be degraded by cellulolytic enzymes to glucose. Gnansounou et al. [4] compared four options for sweet sorghum conversion in which white sugar or ethanol was produced from the juice and the bagasse was burned or converted to ethanol. In this study, the best economic results were achieved when bagasse was converted to fuel ethanol irrespective of what juice was processed for.

The aim of this study was to test Hungarian sweet sorghum as feedstock for ethanol production. Since conventional white sugar production is based on sugar beet in Hungary, the potential of the whole sweet sorghum crop was investigated. Sweet sorghum juice samples harvested at different dates were evaluated upon their sugar content and fermentability. Steam pretreatment of the bagasse was tested at four experimental sets after SO_2 impregnation. The efficiency of the process was evaluated by enzymatic hydrolysis of the whole slurry and the separated fiber. Low substrate concentration was used to minimize product inhibition.

Materials and Methods

Figure 1 shows the schematic representation of the experimental process in that two options of the pentose fraction utilization, which are advantageous for ethanol production, are indicated.

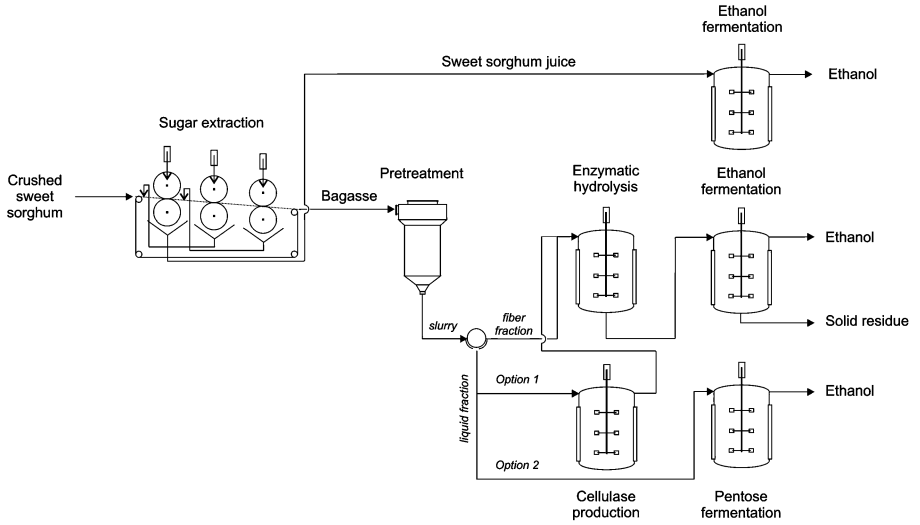

Fig. 1 Schematic diagram of the sweet sorghum utilization for ethanol production

Sweet Sorghum

Hungarian sweet sorghum variety "Berény" was cultivated at Research Institute, Karcag (Centre for Agricultural Sciences and Engineering, University of Debrecen, Hungary) in 2006. This variety is a double-cross sweet sorghum hybrid with a yield of 60–80tons of fresh stem per hectare that is mainly processed to silage. The ratio of stem, leaves, and panicles was found to be about 76%, 16%, and 8%, respectively. Sweet sorghum was harvested at different dates to monitor the change in the sugar content (Fig. 2). Sweet sorghum juice was extracted from the fresh stem with leaves on site. In some cases, extraction of the sugar juice was achieved from the leaf and panicle free stem in order to see if there is any effect of leaf removal on the fermentability of the juice. Solid residue (hereafter called bagasse) was collected, chopped, milled, and dried at 50 °C to 85–90% dry matter content (DM).

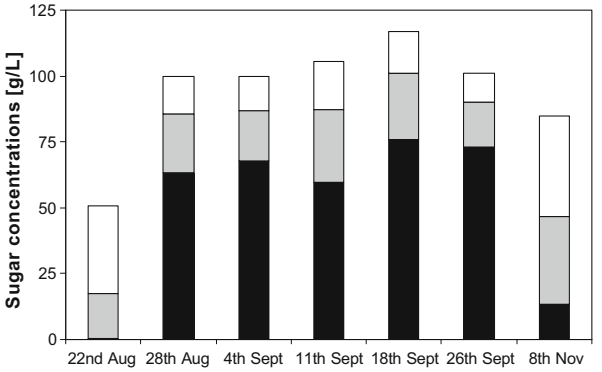

Fig. 2 Sugar concentrations in sweet sorghum juice depending on the harvesting date (sugar extracted from the stalk with leaves on). *Black bars*: sucrose, *gray bars*: glucose, *white bars*: fructose

Steam Pretreatment

Bagasse samples from stem with leaves harvested, chopped, and pressed in September were mixed and steam-pretreated all together at the Department of Chemical Engineering, Lund University, Sweden. The material was steamed at atmospheric pressure for 1h in order to reach 50% moisture content and then impregnated with 2% SO_2 (based on moisture content) in plastic bags for 30 min. Steam pretreatment was performed in a reactor with 10-L working volume [11]. Temperature was set and maintained by injection of saturated steam. Based on previous results reported from sugar cane bagasse pretreatments [12], four experimental settings of steam pretreatment were tested varying the temperature and residence time: 180 °C 10 min, 190 °C 5 min, 190 °C 10 min, and 200 °C 5 min. When the desired residence time on pretreatment temperature was reached, pressure was released and the material was collected in a cyclone. Slurry was divided into two parts: one was used as "whole slurry" in enzymatic hydrolysis and the other part was separated into fiber and liquid fraction. Supernatant of the pretreated slurry was analyzed for sugar and inhibitor content. Dilute acid hydrolysis was performed to determine the total sugar content of the liquid fraction as well. Fiber fraction of the pretreated material was washed with triple amount of hot distilled water to remove the solubilized sugars and inhibitors, and then the washing liquid was removed by filtration. Washed fiber was analyzed for carbohydrate, lignin, and ash content.

Analysis

Lignin and carbohydrate content of raw and pretreated materials were analyzed using NREL protocol [13] with some modification. The amount of oven-dried (105 °C) samples was 0.5 g which was hydrolyzed with 2.5 mL 72% sulfuric acid at room temperature for 2 h. After the reaction time was reached, this mixture was diluted with 72.5 mL distilled water, and the hydrolysis was continued at 121 °C for 60 min. The samples were filtered through G4 glass filter crucibles. The remaining lignin on the filter was dried at 105 °C, weighted, and placed in furnace at 550 °C for 6 h. The Klason lignin content was taken as the ash-free residue after hydrolysis. Ash content was determined gravimetrically. Samples were placed in furnace at 550 °C for 6 h, then the ash content was calculated from the weight loss of samples.

Concentration of the sugar monomers (glucose, xylose, and arabinose) in the supernatant was measured using high-performance liquid chromatography (HPLC). Total carbohydrate content in the supernatant of the pretreated slurry was determined after dilute acid hydrolysis which was performed in test tubes in 5-mL reaction volume with 4% sulfuric acid concentration at 121 °C for 10 min [14].

High-Performance Liquid Chromatography

Liquid samples from pretreatment, total sugar determination, raw material analysis, enzymatic hydrolysis, and fermentation upon their monomer sugar, inhibitor, and/or ethanol concentrations were determined with a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) using an Aminex HPX-87H column (Bio-Rad, Hercules, CA, USA) at 65 °C. The eluent, 5mM H_2SO_4, was used at a flow rate of 0.5 mL/min. Sugar content of the sweet sorghum juice (sucrose, fructose, and glucose) was analyzed with the same HPLC system on Aminex HPX-87P column (Bio-Rad) at 85 °C using ultrapure water as eluent at a flow rate of 0.6 mL/min. Concentration of carbohydrates and inhibitors were detected upon their

refractive index. All samples were filtered through a 0.2-μm pore size filter before analysis to remove solid particles.

Yeast Cultivation

Ordinary baker's yeast (*Saccharomyces cerevisiae*, Lesaffre, Budapest, Hungary) was used in ethanol fermentation. Before fermentation, yeast culture was prepared for inoculation at 30 °C in a 100-mL volume in 750-mL Erlenmeyer flask on an orbital shaker at 350 rpm. Nutrient solution contained 2.5 g/L yeast extract, 5 g/L peptone, 1 g/L KH_2PO_4, 0.3 g/L $MgSO_4$, 2 g/L NH_4Cl, and 50 g/L glucose to which 2 g pressed yeast was added. After 20 h of cultivation, yeast cells were centrifuged and washed two times with distilled water.

Ethanol Fermentation

Ethanol fermentation was carried out at 30 °C in 250-mL screw-capped glass bottles supplied with magnetic stirrers in a 100-mL reaction volume. Sweet sorghum juice and enzymatically hydrolyzed bagasse samples were used as medium without any additional nutrients. After setting the pH to 5.0, bottles were inoculated with the above-described yeast starter culture to a final concentration of 0.5 g DM/L. Fermentation was performed using an online fermentation monitoring system, which is based on measuring the CO_2 production originally described by Veiga et al. [15]. Ethanol fermentation experiments were run for 24 h in duplicates.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed at 50 °C, pH 4.8 (0.05M sodium acetate buffer) using overhead stirring at 250-rpm average agitation speed in 1 L glass bottles. Experiments were carried out in 500-g batches using a substrate concentration of 2% DM both with the whole slurry after pretreatment and with the separated and washed fiber fraction.

Commercial enzyme solutions, Celluclast 1.5 L and Novozym 188 (Novozymes A/S, Bagsvaerd, Denmark) were used. Celluclast 1.5 L is a complete *Trichoderma* cellulase mixture containing exo- and endocellulases, β-glucosidases, and hemicellulases. Novozym 188 is an *Aspergillus* β-glucosidase preparation. Enzyme activity of Celluclast 1.5 L was determined as 101 FPU/mL overall cellulase and 34 IU/ml β-glucosidase. Novozym had a β-glucosidase activity of 435 IU/mL. Filter paper activity measurement was carried out according to Mandels et al. [16], with the modification that an enzyme dilution releasing 1mg glucose was used. β-glucosidase activity was measured according to Berghem and Petterson [17]. Enzyme loadings of 20 FPU/g dry substrate cellulase and 20 IU/g dry substrate β-glucosidase were used. Samples were taken after 0, 2, 4, 8, 24, and 48 h to monitor the hydrolysis. Hydrolysis experiments were run in duplicates on whole slurry and triplicates on washed fiber.

Results and Discussion

Sweet Sorghum Juice

The juice of sweet sorghum is composed of sucrose, glucose, and fructose. In juice sample obtained at the first harvesting date (22nd August), the overall sugar concentration was

rather low (50 g/L) and fructose was found to be the dominant sugar in the juice (65%); only a trace amount of sucrose was detected (Fig. 1). The increase of sucrose content occurs in the reproductive phase of the orthogenesis, especially after the heading stage which is the formation of panicle and its emergence from the boot. Sucrose accumulation in sorghum begins after cessation of internodal elongation obviously making the shift of these culm parts to storage sinks [18]. In the latter samples—except those harvested in November—sucrose was found to be the dominant sugar (57–72%), and in all cases, the amount of glucose was higher than the amount of fructose, which correlates with previously reported results [19]. In the samples without leaf removal, harvested in September, the overall sugar concentrations were found to be between 100 and 117 g/L, as is shown in Fig. 2. Significantly less sugar was observed in the November-harvested samples; probably, sorghum stalks started rapid deterioration after the first frost [20], which was in the middle of October 2006 in Hungary.

Ethanol Fermentation from Sweet Sorghum Juice

In 24-h fermentation experiments, most of the ethanol was produced in the first 8h in all cases.

In pre-experiments, effect of nutrient addition, such as KH_2PO_4, $MgSO_4$, and NH_4Cl, was tested on the yield of ethanol fermentation from the juice (data not reported). Even though Laopaiboon et al. [5] observed that efficient ethanol production from sweet sorghum juice requires nitrogen source supplement, the addition of these nutrients to the fermentation medium did not enhance the ethanol yield by *S. cerevisiae* in our experiments.

Ethanol concentrations and yields using sweet sorghum juice extracted with and without leaf removal are shown in Table 1. There was no significant effect of leaf removal on the ethanol yield; 0.42–0.45 g ethanol was fermented from 1 g sugar. There was a significant difference in the sugar content of the samples extracted in different ways; samples extracted without the leaves showed approximately 20% higher sugar content.

Composition of Sweet Sorghum Crop

Table 2 shows the composition of the sweet sorghum whole crop samples harvested in September at two different dates. Besides water-soluble sugars, there are significant amounts of cellulose and hemicellulose. Since these sugar polymers can also be used for ethanol production, utilization of these constituents should be taken into consideration. Total polysaccharide content is even higher (by 30–35%) than the soluble sugar content in

Table 1 Effect of leaf removal on sugar content of sweet sorghum juice and ethanol yield.

Harvest date	Total sugar (g/g)	Ethanol (g/g)	Yield (g/g)
18th Sept.[a]	116.8	49.8	0.426
26th Sept.[a]	101.1	45.0	0.445
18th Sept.[b]	132.0	55.5	0.420
26th Sept.[b]	130.0	54.1	0.416

Results of ethanol fermentations are presented as mean values of two parallels; the relative standard deviation was below 5%.

[a] The fresh stem was extracted with the leaves on

[b] The stem was extracted after removing the leaves

Table 2 Composition of sweet sorghum (whole crop).

Harvest date	Water soluble sugars (%)	Cellulose (%)	Hemicellulose (%)	Total carbohydrate (%)	Lignin (%)	Ash (%)
18th Sept.[a]	7.47	6.25	3.64	9.89	3.08	0.79
26th Sept.[a]	6.87	6.10	3.22	9.32	3.16	0.68
18th Sept.[b]	9.43	6.27	3.69	9.96	3.21	0.38
26th Sept.[b]	8.80	6.26	3.33	9.59	3.29	0.43

Results of carbohydrate and lignin contents are presented as mean values of three parallels; the relative standard deviation was below 5%.

[a] Samples with leaves on

[b] Samples without leaves

the whole plant, which means that ethanol yield could be doubled by utilizing the polysaccharides besides the soluble sugars.

Although the sugar content of the crop without leaves was higher than that of the whole crop (Table 2), it had no effect on ethanol fermentation from the juice (see yield values in Table 1). Since leaf removal is not feasible in industrial scale, this option can be omitted.

The compositions of sweet sorghum bagasse samples achieved at different harvesting dates are presented in Fig. 3. Although there is a slight increase in cellulose content between samples harvested on 22nd August and 26th September, this increase is not significant, and this is the case in the concentration of the other constituents as well over the investigated period. The average content of cellulose, hemicelluloses, and lignin in sweet sorghum bagasse samples obtained after harvesting in August, September, and November were measured as about 34%, 25%, and 18%, respectively. Consequently, the harvesting date can be determined by the sugar content of the juice; the by-product bagasse can be utilized as harvested at any time.

Steam Pretreatment

Sweet sorghum bagasse was pretreated by steam explosion at various parameters. Table 3 shows the composition of the raw material before pretreatment and the solid

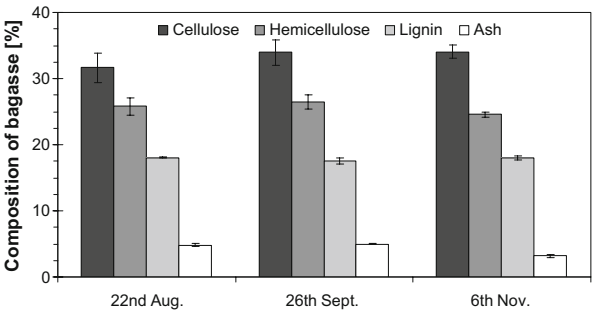

Fig. 3 Composition of sweet sorghum bagasse at different harvesting dates

Table 3 Analysis of data from different pretreated materials: composition of raw bagasse and washed fiber fraction of the pretreated bagasse.

Pretreatment		$\log R_0$	Carbohydrate content (%)			Lignin content (%)
Temperature (°C)	Time (min)		Glucan	Xylan	Arabinan	
Untreated		–	36.25	25.64	2.04	18.6
180	10	3.36	50.70 (96.0%)	20.62 (63.1%)	2.11	22.9
190	5	3.35	51.74 (96.1%)	18.17 (53.9%)	2.12	22.5
190	10	3.65	58.32 (95.9%)	12.75 (25.9%)	2.07	24.6
200	5	3.64	55.73 (93.7%)	13.15 (28.3%)	1.93	25.2

Results are presented as mean values of three parallels; the relative standard deviation was below 5%. Data in parenthesis show the residual percentages of glucan and xylan based on their mass in raw bagasse

fraction of the pretreated material. Steam explosion was characterized with the severity factor (Eq. 1) introduced by Overland and Chornet [21].

$$R_0 = t \times \exp\left(\frac{T - 100}{14.75}\right) \tag{1}$$

where t is time in minutes and T is temperature in °C. Severity factor is the logarithm value (base 10) of R_0. According to these values, the severities of the two used milder and two harsher pretreatment settings seem to be very similar.

Glucan ratio of the substrate has increased significantly during the pretreatment. Milder pretreatment conditions resulted in 40–43%, while harsher experimental sets caused about 54–61% increase in glucan content. At milder pretreatment conditions, xylan content decreased by 20–30%, while at harsher sets, about 50% decrease was observed due to the acid-catalyzed hydrolysis of the hemicellulose fraction that results more accessible cellulose in the enzymatic hydrolysis. This is equivalent to 70-75% solubilisation of the initial xylan content of the raw material. As the hemicellulose ratio has been reduced significantly, lignin content of the fiber has increased due to the pretreatment. The initial 18% lignin content increased to 23% after milder and to 25% after harsher pretreatment.

The ratio of glucan to xylan in the fiber fraction has increased with the severity of the pretreatment. In the raw bagasse, the ratio of these compounds was 1.41, which has increased to 2.45 and 2.84 after the milder and to 4.58 and 4.24 after the harsher pretreatments (data are in the same sequence as the pretreatment settings in Table 3, respectively). This indicates that significant amount of xylan has been solubilized, while the glucan content has only been degraded to a minor extent. Although the xylan removal can be increased under harsher pretreatment conditions, degradation of glucan is more pronounced using higher temperature and/or longer residence time. Ballesteros et al. [22] observed 92% decrease in xylan content; however, the glucan ratio of the fibers increased only by 19% when sweet sorghum bagasse was steam-exploded at 210 °C for 2 min without acid catalysis. A compromise between the highest xylan and the lowest glucan hydrolysis during pretreatment has to be found especially when the pentose fraction is separated and further used for ethanol production.

Sugar and inhibitor content of the pretreatment supernatants are shown in Table 4. All sugar concentrations in the liquid fractions increased with the severity; the highest values were measured in the supernatant at the pretreatment parameters of 200 °C and 5 min. The monomer glucose contents varied between 3.6 and 7.8 g/L, while the total glucose concentrations obtained after dilute acid hydrolysis varied between 13.0 and 18.0 g/L. The xylose and water-soluble xylan oligomers were released at higher extent due to the harsher

Table 4 Analysis data from different pretreated materials: sugar and inhibitor content of the liquid fraction from the pretreated slurry.

logR_0	Concentration (g/L)									
	Glucose	Cellobiose	Total glucose[a]	Xylose	Total xylose[a]	Arabinose	Acetic acid	HMF	Furfural	Formic acid
3.36	3.55	1.31	13.00	6.41	20.63	3.03	1.656	0.124	0.226	3.295
3.35	5.38	1.41	16.62	15.12	33.2	4.76	2.762	0.179	0.405	4.161
3.65	6.98	2.55	14.78	20.82	36.38	4.67	4.954	0.359	1.002	3.564
3.64	7.77	2.95	17.96	22.97	44.14	5.53	5.394	0.392	0.862	4.505

Results are presented as mean values of three parallels; the relative standard deviation was below 5%.

[a] After dilute acid hydrolysis

pretreatment; the xylose monomer concentration was between 6.4 and 23.0 g/L, and the total xylose content varied between 20.6 and 44.1 g/L. The liquid fraction contains significant amount of carbohydrates (total glucose and xylose content together takes up to 62 g/L), which makes this fraction a suitable material for other fermentation processes after separation.

Since inhibitors can interfere with microbial growth, which might cause a problem in the ethanol fermentation step, their concentrations were also measured in the supernatant. Furfural and 5-hydroxymethyl-furfural (HMF) are the degradation products of pentoses and hexoses, respectively. The amounts of these compounds were found to be below the inhibiting concentration [23] at all studied conditions. Formation of formic acid correlated with the residence time instead of the severity factor (logR_0). Applying short residence time, the concentration of formic acid was higher. Formic acid is formed when furfural and HMF are broken down [24]. The reason for the decrease in formic acid concentration when longer residence times are applied may be that formic acid can react with other compounds at these conditions in a low-rate reaction. Formation of acetic acid correlated rather with the severity factor; harsher pretreatment conditions resulted in higher acetic acid concentrations. Acetic acid is released from the acetylated hemicelluloses during pretreatment.

Enzymatic Hydrolysis

Enzymatic hydrolysis was performed both on the whole pretreated material and the separated washed fiber fraction. The advantage of separation of the solid and the liquid fractions after pretreatment is that the xylose-rich liquid fraction can be used for pentose fermentation, cellulase enzyme production [25], or transformation to various valuable products through bio- and chemical conversions. Figure 4 shows the hydrolysis curves of pretreated sweet sorghum bagasse using the whole slurry, i.e., without separation of the fiber and liquid fractions. The achieved final glucose concentration values were around 7 g/L in the case of the two milder pretreatments and between 11–12 g/L of the two harsher-pretreated bagasse samples. As the xylan content of the raw material is rather high, the xylose concentration in the hydrolysates was also considerable, around 4 g/L from the bagasse samples pretreated at milder and between 6 and 6.5 g/L from the samples pretreated at harsher conditions. These results show that xylose amounts to one third of the total sugar content in the hydrolysates; thus, efficient ethanol fermentation requires a microorganism able to ferment both sugars. Although recombinant xylose-utilizing yeasts have been developed, lower xylose concentration in the hydrolysate is favorable. Xylose is metabolized only after consumption of all glucose, since uptake of both sugars are through

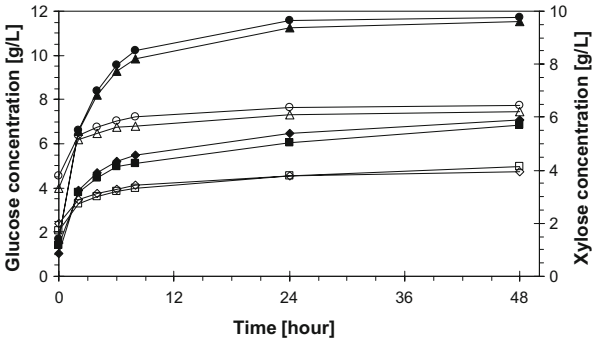

Fig. 4 Glucose (*open symbols*) and xylose (*closed symbols*) concentrations in hydrolysis of pretreated bagasse samples using 2% DM of whole slurry. Pretreatment conditions: 180 °C 10 min (*square*), 190 °C 5 min (*diamond*), 190 °C 10 min (*triangle*), 200 °C 5 min (*circle*). Results are presented as mean values of two parallels; the relative standard deviation was below 5%

the glucose transporters, which have more than 100-fold lower affinity for xylose compared to glucose [26].

Figure 5 shows the final (48h) conversions of the whole slurry (white bars) and the washed fiber hydrolysis experiments (gray bars). Conversion is expressed as a percent of the released glucose compared to the glucose equivalent of the total glucan content of the substrate. While 16% conversion was observed (data not reported) after 48 h enzymatic hydrolysis of untreated sweet sorghum bagasse, with 180 °C 10 min and 190 °C 5 min pretreatment, 48% and 55% conversions of the whole slurry were achieved, respectively. Using harsher pretreatment conditions (190 °C 10 min and 200 °C 5 min), 83% and 86% conversions were reached, respectively. Washed fibers from bagasse pretreated at milder conditions (180 °C 10 min and 190 °C 5 min) reached 45% and 53% conversions, respectively. After harsher pretreatment (190 °C 10 min and 200 °C 5min) and washing step, 89% and 92% conversions were reached in the case of separated fibers, respectively. In contrast to the results using mild conditions, in the case of harsh pretreatments, conversions of the washed fibers were about 7% higher compared to the results obtained from whole slurry hydrolysis. Pretreatment of the substrate at the mild experimental settings (180 °C 10 min and 190 °C 5 min) have resulted in low enzymatic digestibility of the cellulose fibers, while harsher pretreatment conditions proved to be sufficient to increase degradability significantly. Increasing the temperature from 180 °C to 190 °C with 10-min residence time resulted in 44% increase in the final cellulose conversion of the washed fiber. Increasing the temperature from 190 °C to 200 °C at 5-min residence time, 39% improvement in the conversion was obtained. Comparing the effect of the residence time at 190 °C, 36% higher conversion was observed by increasing the pretreatment time from 5 to 10 min.

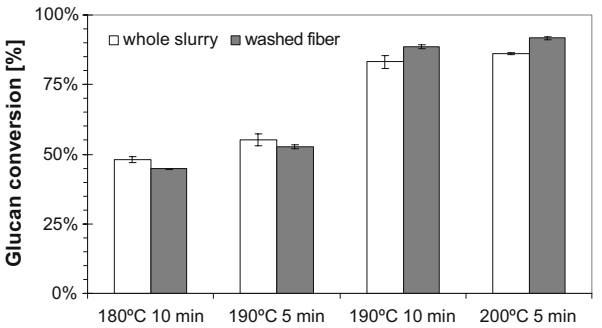

Fig. 5 Effect of liquid fraction separation on the hydrolysis of steam-pretreated sweet sorghum bagasse using 2% DM substrate concentration in 48-h hydrolysis. Conversion results are presented in percentage of the theoretical yield calculating with all the potential glucose in the pretreated material

Conclusions

The objective of this work was to test Hungarian sweet sorghum as a possible feedstock for ethanol production. It was found that sweet sorghum can be harvested over at least a 1-month period with high amount of sugars in the stem. The juice extracted from the harvested plant was found to be an appropriate feedstock for ethanol fermentation. The effect of leaf removal proved to be negligible from the aspect of the ethanol yield achieved in fermentation by ordinary baker's yeast. The solid residue, bagasse, was investigated for possible utilization as lignocellulosic raw material for alcohol production. Steam pretreatment of SO_2-impregnated bagasse has been tested at different residence times and temperatures. Two of the used conditions resulted in easily digestible material by cellulosic enzymes. At 190 °C 10 min and 200 °C 5 min pretreatment parameters, 89% and 92% cellulose conversion of the separated washed fiber fraction could be achieved, respectively.

Applying separation step, cellulose fibers can be separated from inhibitory components of yeast ethanol fermentation, such as furfural, HMF, acetic acid, or formic acid. The advantageous effect of inhibitor removal in hydrolysis was observed only at harsh-condition-pretreated substrates where the final conversions increased by about 7%. Fiber separation followed by enzymatic digestion results in a readily fermentable hydrolysate (in our experiments, around 80–90% glucose to ethanol yields were achieved by *S. cerevisiae*; data not shown). Moreover, applying separation step before enzymatic hydrolysis, pentoses presented in the liquid fraction can be processed to several valuable products. This might be a basis for a lignocellulose biorefinery.

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